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Jarisch-Herxheimer reaction in complement-depleted rabbits

Histological and immunofluorescence studies of early cutaneous lesions

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SUMMARY The possible role of complement in the pathogenesis of the Jarisch-Herxheimer reaction was assessed in cutaneous syphilitic lesions in two groups of rabbits treated with penicillin; in one group complement was depleted before penicillin therapy. Serial biopsy specimens were similar histologically in both groups. The activation of the complement pathways did not seem to play a role in the pathogenesis of early cutaneous syphilitic lesions in rabbits during the Jarisch-Herxheimer reaction.

Introduction

The Jarisch-Herxheimer reaction (JHR) was first described in 1895 by Jarisch and in 1902 by Herxheimer and Krause as a transient exacerbation in the appearance of mucocutaneous lesions in early syphilis after treatment with mercury had been started. The reaction was subsequently reported after the beginning of treatment of syphilis with arsenic, bismuth, penicillin, and immune serum.[1 2] The JHR has also been described in other spirochaetal diseases, some bacterial infections, and African trypanosomiasis.[3]

The clinical manifestations of the JHR include exacerbation of the lesions, fever, rigors and, in more severe reactions, headache, sore throat, malaise, myalgia, arthralgia, nausea, and vomiting.[4] In early syphilis the clinical reaction starts about four hours after treatment has begun, reaches a peak at about eight hours, and subsides by 16 hours.[2] The JHR occurs in both the sero-negative and sero-positive phases of primary syphilis and in secondary syphilis.[5]

Several mechanisms have been suggested for the pathogenesis of the JHR. Herxheimer attributed it to the release of treponemal breakdown substances or endotoxin.[2] A lipopolysaccharide has been identified in *Treponema pallidum* which could account for the endotoxin.[6 7] Endotoxin has been detected by the limulus lysate assay in the plasma of patients with the JHR in both louse-borne relapsing fever and syphilis.[3 8] Gelfand *et al* reported the presence of endotoxin by the limulus lysate assay together with modestly decreased C3 during the JHR, while the total haemolytic activity, C4, and properdin were unchanged. This suggested that activation of the alternate complement pathway by the lipopolysaccharides was the cause of the JHR rather than antigen-antibody complexes.[8] Fulford, however, found C3, C4, C6, C7, CH50, and C1 inhibitor were depressed, which suggests activation of the classic complement pathway and does not support the role of the endotoxin as the direct aetiological mechanism.[9]

The skin lesions of secondary syphilis are characterised by endothelial proliferation and a perivascular inflammatory infiltrate mainly consisting of lymphocytes and plasma cells.[10] In the JHR transient acute inflammatory infiltrates are superimposed on the lymphocytic vasculitis.[2] Specific antigen-antibody complexes have been observed in early syphilitic nephritis and specific antitreponemal antibodies have been demonstrated in the cutaneous lesions of early syphilis in both man and rabbits.[11-13]

In non-syphilitic vasculitis of both necrotising and lymphocytic types biopsies of skin lesions have demonstrated immunoglobulins or complement components or both fixed to vessel walls, which may

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implicate activation of the classic or alternate complement pathways by either immune complexes, endotoxin, or other substances.[14] The intradermal inoculation of *T pallidum* into rabbits results in changes similar to the naturally occurring disease in man; the rabbit is, therefore, a useful animal model for the study of experimental syphilis.[15 16] This study was designed to assess the possible role of complement in the cutaneous syphilitic lesions of rabbits during the JHR.

Material and methods

RABBITS

Twelve albino rabbits, weighing 2-3 kg each, were caged separately, fed an antibiotic-free diet with free access to water, kept at 18°C, and observed for two weeks before inoculation. A rabbit inoculated intratesticularly with live *T pallidum* was obtained from the Venereal Disease Research Branch of the National Communicable Disease Center in Atlanta, Georgia.

INOCULATION

An inoculum was prepared by adding 60 ml of phosphate-buffered saline (PBS) pH 7·2, without bacteriostatic agents, to two emulsified testicular lesions. The supernatant of this suspension contained 3×10^7 motile treponemes per ml, and 0·2 ml was injected into each of 12 sites on the shaved dorsal skin of each rabbit. Each rabbit was also inoculated intravenously with 2 ml of the supernatant.

PENICILLIN TREATMENT

The inoculated rabbits developed erythematous macules and papules within a week, which became infiltrated nodules by the tenth day. On the fourteenth day the rabbits were divided into two groups. The first group, which served as controls, each received a single intramuscular injection of procaine penicillin 60 000 units/kg. The second group was rendered complement deficient before the penicillin injection by an intravenous injection of cobra venom factor (Cordis Laboratory, Miami, Florida, USA (lot No 80696)), 200 units/kg, 18 hours before penicillin therapy.[17] Injection of 185 units/kg of this preparation intravenously into the rabbits lowered the haemolytic complement activity to almost zero at 17 hours.

LIGHT AND FLUORESCENT MICROSCOPY

After local infiltration with 1% lidocaine, 4-mm punch biopsy specimens were obtained from the cutaneous nodules shortly before treatment with penicillin (0 hr), and at 4, 8, 16, 24, and 48 hours after treatment. Each biopsy specimen was bisected. One half was fixed in 10% buffered formalin solution, embedded in paraffin, cut into 5-μ thick sections and stained with haematoxylin and eosin for examination by light microscopy.

The other half was immediately frozen in liquid nitrogen. Six-micron thick frozen sections were cut from these specimens and stored at −80°C until processed. These frozen sections were stained with a commercially prepared fluorescein isothiocyanate (FITC)-labelled goat anti-rabbit immunoglobulin (Hyland, California) and FITC-labelled anti-rabbit complement (C3) (Cappel Labs, Cochranville, Pennsylvania) by a standard direct immunofluorescence technique.[14] Anti-C3 activity was confirmed by immunodiffusion. Frozen sections of healthy rabbit skin served as negative controls. The preparations were then mounted, sealed, and examined with a fluorescence microscope with 200 watt mercury arc lamp, exciter filter 702, and barrier filter OG-1 and photographed.

At the prescribed intervals the rectal temperature of each rabbit was also recorded.

The degree of polymorphonuclear leucocyte (PMNL) infiltration was graded as: 1, 0-10 PMNL/high-power field (hpf); 2, 11-20 PMNL/hpf; and 3, >20 PMNL/hpf). Six high-power fields, which were randomly selected in two sections of each biopsy specimen, were examined.

Results

TEMPERATURE

The temperature was raised minimally after the injection of penicillin and was similar in both groups of rabbits (fig 1).

HISTOLOGICAL CHANGES

Inflammatory infiltrates

Pre-treatment specimens showed a preponderant accumulation of lymphocytes, plasma cells, and histiocytes. The number of polymorphonuclear leucocytes, which was small (<10/hpf) in 0-hour specimens, increased in the specimens subsequently obtained, and slowly decreased after 24 hours (fig 1). The 48-hour specimens contained fewer polymorphonuclear leucocytes; these were found in the dermis and, in some specimens, were associated with nuclear dust. The inflammatory infiltrates of the two groups of rabbits showed similar changes (figs 2-6).

Oedema

This was present in minimal to mild degrees before treatment, increased rapidly to moderate or severe degrees in the four-hour and eight-hour specimens, persisted through the 24-hour specimens, and subsided thereafter. The degree of oedema was similar in the two groups.

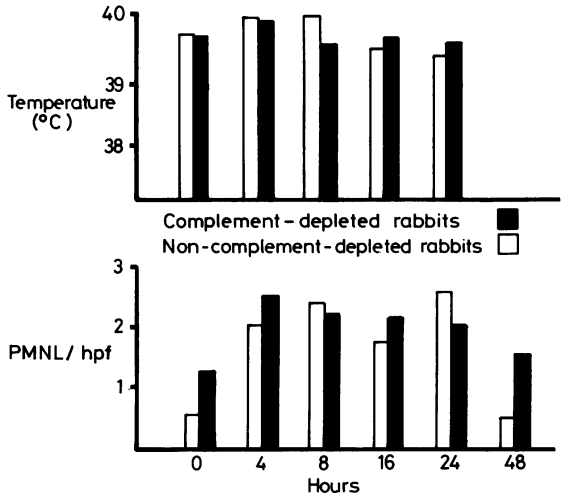

FIG 1 *Sequential changes in temperature and polymorphonuclear leucocyte (PMNL) infiltrates in the cutaneous lesions during the Jarisch-Herxheimer reaction in the complement-depleted rabbits. PMNL numbers are indicated by 1, 0-10 PMNL/hpf; 2, 11-20 PMNL/hpf; and 3, >20 PMNL/hpf.*

IMMUNOFLUORESCENCE STUDIES

Except for some non-specific diffuse evidence of immunoglobulins, which correlated with the oedema, there was no specific or pronounced deposition of immunoglobulins on the blood vessel walls or at the dermal-epidermal junction. C3, which was absent in the 0-hour specimens, remained so throughout the study. There was no difference in the immunofluorescence findings between the two groups of rabbits.

Discussion

Sheldon and Heyman[2] found transient acute inflammatory changes in the syphilitic lesions of both human subjects and rabbits 4-6 hours after the beginning of treatment. Dilation of the capillaries and venules was followed by endothelial swelling and migration of leucocytes through vessel walls into the surrounding oedematous tissues. The inflammation subsided within 18 hours without pronounced degenerative changes of the vessel walls or intravenous thrombus formation. Histological findings in biopsy specimens obtained at 72 hours resembled those of the pretreatment specimens. In a number of

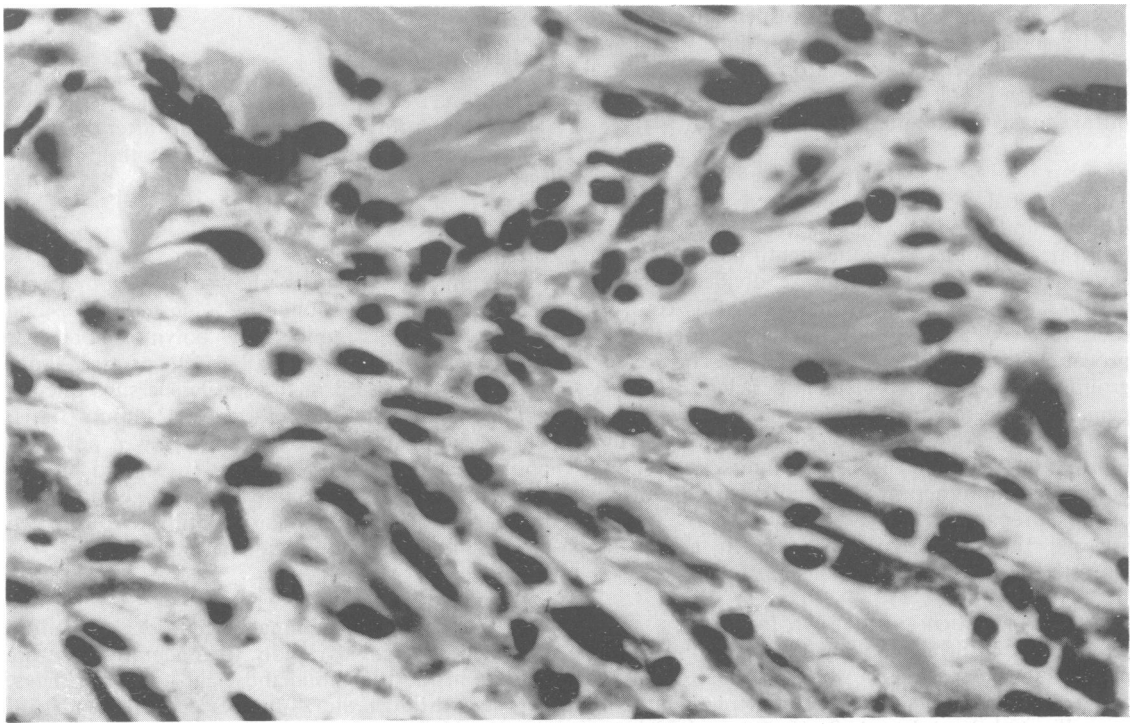

FIG 2 *Histological changes in cutaneous lesions before penicillin therapy in a complement-depleted rabbit. Note thickening of blood vessels and the lymphoplasmocytic infiltrate. (Haematoxylin and eosin, × 400)*

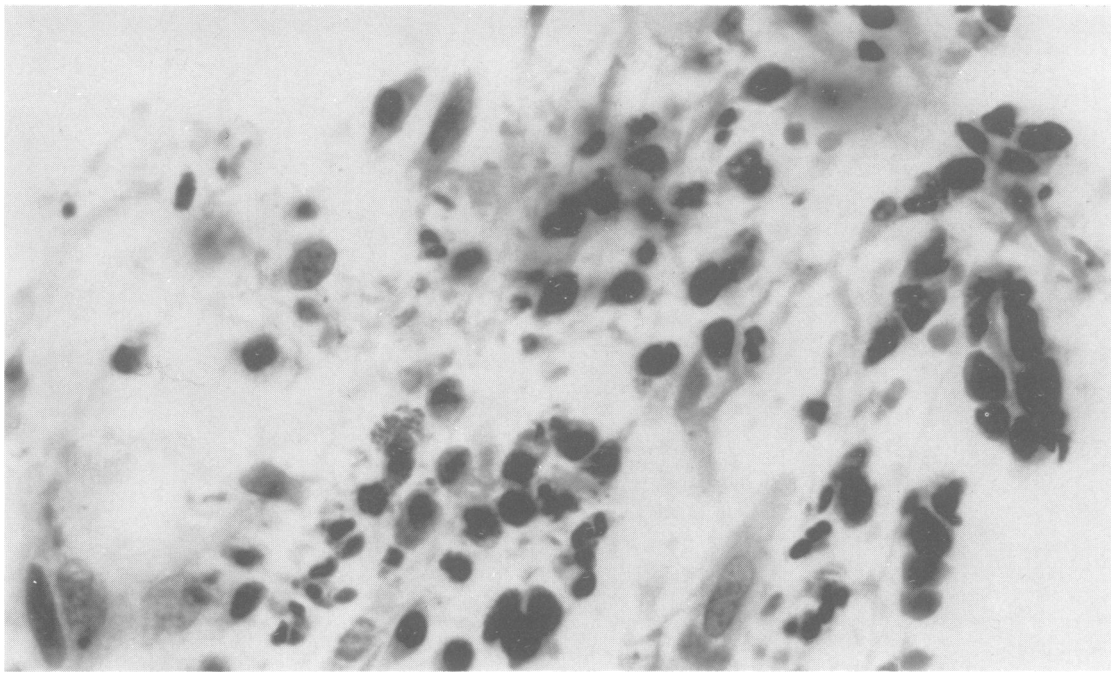

FIG 3 *Histological changes in cutaneous lesions four hours after penicillin therapy in a complement-depleted rabbit. Note oedema and the presence of polymorphonuclear leucocytes in the dermis. (Haematoxylin and eosin, × 400)*

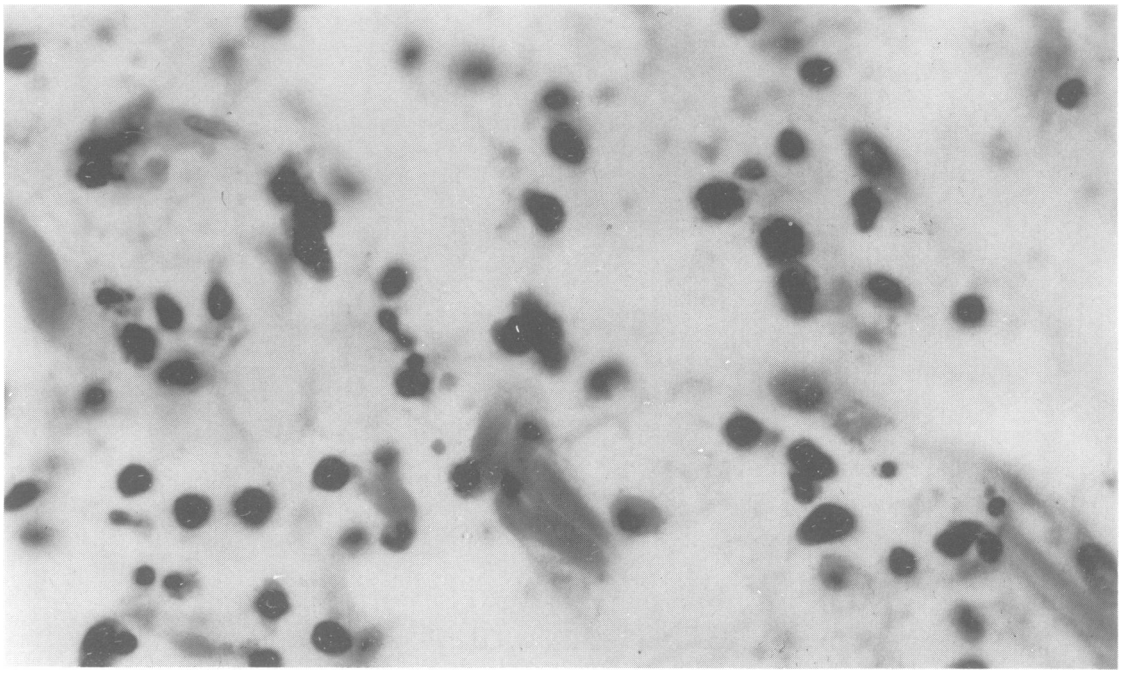

FIG 4 *Histological changes in cutaneous lesions eight hours after penicillin therapy in a non-complement-depleted rabbit. Note oedema and increased numbers of PMNL compared with fig 3. (Haematoxylin and eosin, × 400)*

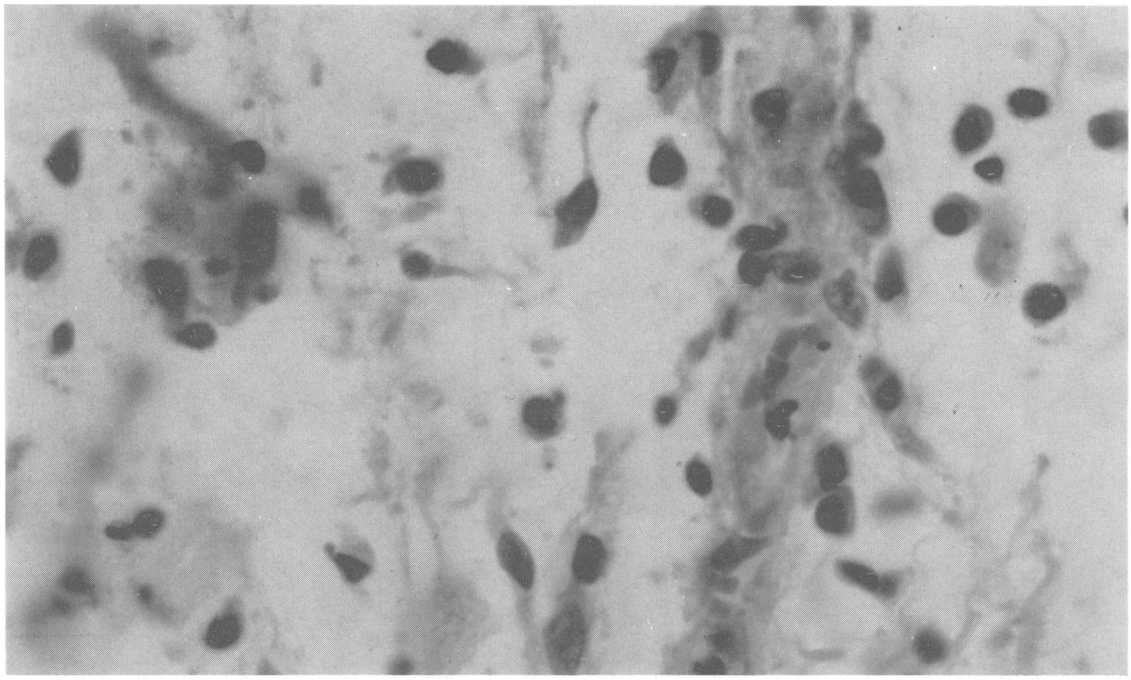

FIG 5 *Histological changes in cutaneous lesions 24 hours after penicillin therapy in a complement-depleted rabbit. Note disruption of collagen architecture. (Haematoxylin and eosin, × 400)*

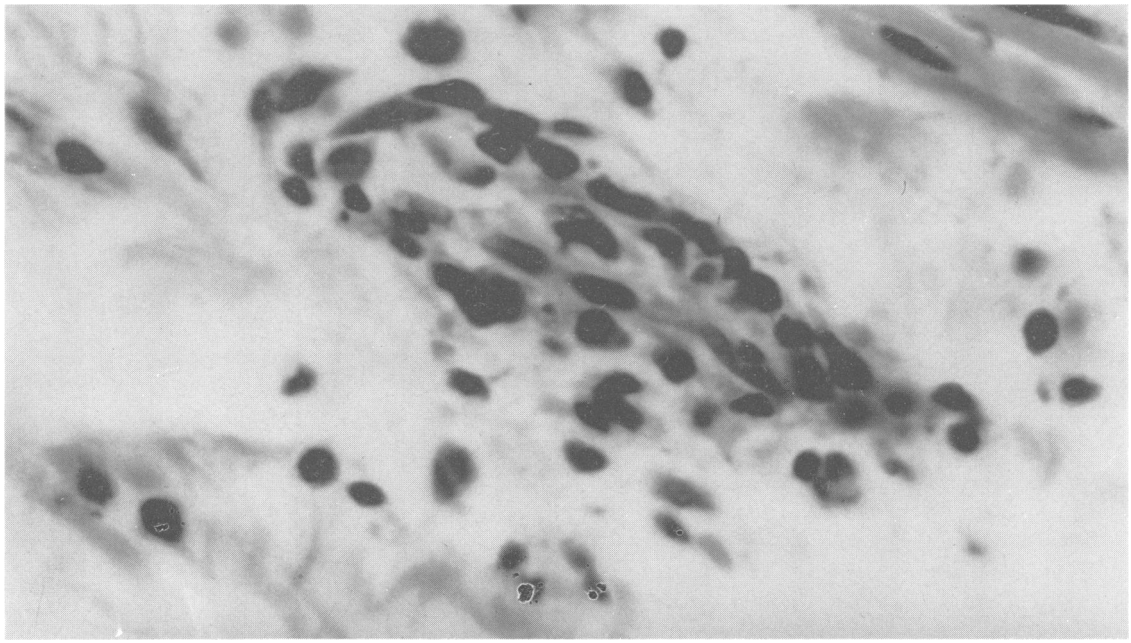

FIG 6 *Histological changes in cutaneous lesions 48 hours after penicillin therapy in a complement-depleted rabbit. Note preponderance of plasma cells, decreased numbers of PMNL, and oedema in the dermis. (Haematoxylin and eosin, × 400)*

patients with equivocal symptoms of the JHR, similar histological changes were detectable.[2] Thus the histological findings are a more sensitive indicator of the JHR especially in the clinically undetectable forms of this reaction.[2]

Our findings confirm those of previous studies in rabbits which indicated that histological changes in the syphilitic lesions were a more sensitive indicator of the JHR than the clinical manifestations. Although the temperature changes were minimal, the histological sections showed an increase in polymorphonuclear leucocytes for 24 hours after the JHR had started.

The inflammatory infiltrate in the group of rabbits with depleted complement did not differ from the infiltrate in the control group. Immunofluorescence studies of the frozen sections in the two groups did not detect the C3 component of complement in the lesions from either group.

In this study the activation of the complement pathways, as indicated by the deposition of C3, did not seem to play a major role in the histological changes of the cutaneous lesions of early syphilis in rabbits during the JHR.

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